

Introduction

Specific animal tests are currently legally mandated to ensure the safety of industrial chemicals, pesticides (plant protection products and biocides), food additives and food contact materials, pharmaceuticals, etc., before they can be marketed in the European Union. The process of animal testing in the UK is controlled through strict licensing regulations and animal welfare considerations are given considerable attention.

Alternative techniques are being developed to replace much of the present animal testing and include *in vitro* assays, use of structure-activity relationships and computer modelling. The 7th Amendment to the EU Cosmetics Directive 76/768/EEC will phase in a ban (2004 -2013) on animal testing of personal care products and ingredients and will require the use of alternative testing methods where these are available and have been validated. It should be noted that 'natural' chemicals, which may be promoted as alternatives to synthetic chemicals, are not necessarily safer; some of these naturally occurring chemicals are toxic and may not have been tested for their potential health effects.

The 3Rs Principles

The 3Rs principles of reduction, refinement and replacement are the cornerstone of all animal experimental design and should be applied, wherever possible, by reducing the numbers of animals used in testing, refining the tests so as to minimise potential pain, suffering or distress and replacing animal tests where non-animal methods are available.

Efforts have been made to refine longer-term studies, such as combining repeat dose and reproduction studies, in order to reduce overall animal usage and to maximise the number of observations performed in a study. Efforts have also been made to provide improved group housing and an enriched environment for experimental animals. More effort needs to be spent on optimising testing strategies to ensure that only relevant and necessary testing is performed. This will require regulatory bodies to take a lead in funding such research.

The EU is funding the European Centre for the Validation of Alternative Methods (ECVAM) to animal experimentation which aims to encourage the international development and acceptance of alternatives. The UK Government, together with industry and other bodies, is funding a centre to promote and apply the 3Rs principle (NC3R's – National Centre for the Replacement, Refinement and Reduction of Animals in Research). The European Partnership for Alternative Approaches to Animal Testing (EPAA), a joint initiative from the European Commission and a number of companies and trade federations is also conducting research into alternative methods and their validation.

ECVAM provides scientific and technical advice to EU Commission services, such as to Directorate General (DG) Environment, DG Enterprise, DG Health and Consumer Protection and DG Research, and undertakes projects on validation activities.

ECVAM performs research on the development of advanced testing methods and validation of alternatives to animal tests. Over the past years, ECVAM has increasingly been involved in supporting the implementation of the new EU policies on cosmetics (*Council Directive 2003/15/EC*) and the Registration, Evaluation and Authorisation of Chemicals (REACH) Regulation which calls for the use of animal alternatives and testing strategies as soon as possible. Accordingly, ECVAM has restructured its services by directly targeting the animal tests to be replaced.

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The key areas, in which ECVAM is active include *topical toxicity* (e.g. local adverse effects on skin or eyes), *systemic toxicity* (which requires absorption of the toxicant within the blood and then distribution to the target organ(s) and system(s) which are the place of action (e.g. to the liver, kidneys or the blood-forming system, immune, nervous and reproductive system), *special aspects* (e.g. carcinogenicity, integrated testing, metals toxicity), *ecotoxicity* and *strategic activities* which have relevance to all different kinds of toxicological studies. ECVAM furthermore, covers the following topic areas:

- biologicals and biomaterials
- quality control and safety testing (e.g. vaccines, monoclonal and polyclonal antibodies),
- hormones,
- biocompatibility of medical devices, and
- *in silico* methods (e.g. quantitative structure activity relationships (QSARs)).

One of ECVAM's priorities is to ensure that it is well-informed about the state-of-the-art of non animal test development and validation in relation to particular types of chemicals/products and potential toxic hazards. ECVAM workshops are held to review the current status of various types of alternative tests and their potential uses, and to identify the best ways forward. ECVAM Task Forces focus on more tightly-defined targets. Reports and recommendations of ECVAM workshops and task force meetings are available on the ECVAM website (<http://ecvam.jrc.it/index.htm>).

Alternative Testing Approaches

Considerable investment is needed into research on alternatives. Such research does not currently have a high academic profile so that, most research has been supported by the industrial research community, through in-house research and through organisations such as the Fund for the Replacement of Animals in Medical Experiments (FRAME), NC3Rs and EPAA. Recently, the Home Office has begun to raise the profile of this work in the UK. Alternative techniques cover a number of possibilities, including:

Computer modelling (in silico techniques)

Physiologically-based pharmacokinetic (PBPK) modelling and other computer-based modelling techniques model the absorption, distribution, metabolism and excretion of chemicals in the human body. Currently, they use real physiological and metabolic data, but it should be possible to develop models that use pre-existing physiological data and *in vitro* metabolic information. Computer modelling can sometimes be used to predict the range of responses across a population by simulating the variation in these physiological and metabolic parameters within populations.

Structure-activity relationships

Structure-activity relationships can predict the potential health effects of a chemical from its physico-chemical properties and its structural similarities to other chemicals with known health effects. Important lessons can be learned from the transfer of data from other fields, e.g. mammalian toxicity, ecotoxicity, and fish toxicity. Information collected from human studies on pharmaceutical and personal care product ingredients would also be useful in predicting the effects of chemicals of similar structure. Extrapolation human data and structure activity information can reduce the numbers of animals required.

In vitro assays

These are tests to measure certain health end-points of chemical exposure in a 'test-tube' (*in vitro*), without using animals. Some of these tests have been validated and widely used (e.g. genotoxicity, skin corrosion). Other end-points for reproductive toxicity and chronic toxicity are harder to measure in this way and will require further research. Some progress has been made on embryotoxicity but this is just one part of the reproductive process. The greatest problem and one for which there seems at present to be no possible alternative to animal testing is the study of cancer (both the scientific evaluation of the cancer process and carcinogenicity testing of chemicals) and toxic responses to chemicals that involve the integrated 'whole organism'. However, *in vitro* tests almost invariably focus on single end-points.

Modern technologies, such as the '-omics' (genomics which looks at the effect of chemicals on a whole range of genes, proteomics which look at protein and peptide expression, and metabolomics which looks at metabolites) offer potential for many more *in vitro* tests to become available.

However, before all this can happen, these new tests need to be validated to ensure that they are reliable and useful. The current timescale for validation, acceptance, and implementation of alternative tests is extremely long and needs to be addressed with diplomatic pressure on the EU from the UK and other Member State governments.

Current Limitations

New tests are coming forward and a clear process is now in place, both nationally and internationally, to ensure that they are developed. However, there are two key limitations affecting alternatives to animal tests.

The first is a lack of confidence in the results generated. This is dealt with during development through examinations of robustness and consistency and a comparison of results from the new and existing tests in terms of their 'false positive' and 'false negative' rates, which should be conducted using comparison with human evidence. Even with straightforward 'plug-in' replacement assays (e.g. the mouse Local Lymph Node Assay (LLNA) for skin sensitisation in place of the guinea pig tests, all of these being *in vivo* assays), regulators and possibly industry as well are slow to eliminate a well understood, if not always properly tested, guideline for one they have little experience of.

Once a new test has been identified, the chief problem is the length of time needed for it to gain international acceptance. For example, the time taken from initial development to validated method is often 10-15 years and additional time is needed to gain Organisation for Economic Co-operation and Development (OECD) acceptance and for the publishing of guidelines. The test has to be validated in international trials and international regulators have to agree on its role, the appropriate protocols and the animal testing which can be withdrawn as a result. The trend in regulatory risk assessment has been towards the use of alternatives as 'screens', whereby only positive results obviate the need for the *in vivo* assay. The exception is genotoxicity, where three negative *in vitro* assays are usually deemed to be an adequate demonstration of a lack of genotoxicity for chemicals. Nevertheless, agencies such as ECVAM are aware of the need to push forward with test validations particularly in order to meet the requirements of REACH, and are looking at alternative validation paradigms to reduce the timescales.

The second is the lack of a suitable approach to risk evaluation within which to analyse the results of alternative methods.

The Future

The key drivers for the development of alternatives to animal testing are the moral and ethical issues concerning testing in humans and animals together with the development of scientific knowledge. New testing procedures for existing and newly identified end points will always be required and should be based on the 3Rs.

A key necessity for the future is a new approach to risk evaluation. The current approach uses hazard information based on animal testing as the primary source for a risk evaluation. It also uses a fixed level of risk (in the evaluation, usually expressed as the reference dose – for example an 'acceptable daily intake' in the case of food additives). Any new approach requires a more risk based, and therefore exposure-driven risk evaluation. Classically, this means moving to a process whereby the type of exposure and the 'margin of exposure' (the gap between the dose at which the effect is/might be seen and the exposure level for the appropriate type of exposure) determines the extent of information required. Even if the information on effects is poor, when the gap is sufficient then the risk is not sufficient to justify further quantifying it through conducting a more detailed, usually animal, test. Further, it requires a move from the current system, whereby the animal test is the pivot from which the key effect is derived for human health risk assessment, to a system that can cope with using non animal data (with, if necessary, greater margins of exposure) as the key.

Conclusion

Industry and others are actively developing alternatives that would reduce the need to use animals for toxicity testing, as far as practicable. Greater research effort is needed to develop and validate alternative tests that are acceptable to the OECD. Until these are in place, wherever possible, the 3Rs principles of reduction, refinement and replacement should be applied in reducing the numbers of animals used in testing, refining the tests that are used to reduce potential suffering and replacing animal tests where alternatives are available.